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GENETIC AND NON-GENETIC VARIABILITY IN TEMPERATURE TOLERANCE OF THE COPEPOD *EURYTEMORA AFFINIS* IN FIVE TEMPERATURE REGIMES

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Abstract

Genetic and nongenetic variation in temperature tolerance was measured in populations of copepods grown in two environments varying between 10° and 23°C on 26 day (SW) and 26 week (LW) cycles and in three constant environments at 10°C, 15°C, and 23°C.

Genetic variation was maintained and expressed in both sexes in the cycling environments, but declined in males in the 23°C constant environment, perhaps indicating constant directional selection.

Physiological variation was similar in males and females, in contrast to earlier results, again suggesting an effect of selection. There was evidence of selection for physiological flexibility in males in the 23°C and 15°C environments.

A question arising was why sexual dimorphism in genetic variation and in physiological variation was maintained in nature but reduced, reversed, or even eliminated in the laboratory environment. Random drift was not a plausible explanation.

Introduction

The copepod *Eurytemora affinis* is a seasonally dominant species in the zooplankton of Chesapeake Bay. The greatest densities occur in winter and early spring. Although water temperatures can reach 30°C in summer and 0°C in winter, no resting stages have been found in this species.

Since generation time in *Eurytemora* varies from about 10 days at 23°C to 2 months at 4°C, breeding individuals do not experience the entire 30°C range in temperature. Thus, the species may adapt to temperature variation either partly through changes in gene frequency, progeny being adapted to the temperature at which their parents were selected, or entirely physiologically. Some physiological adjustment is inevitable since individuals experience some variation in ambient temperature, in space if not in time.

Previous workers, such as Marshall and Jain (1968), Levins (1969), and Selander and Kaufman (1973), have suggested that in populations or species with relatively large amounts of physiological variation there is less genetic variation, and *vice versa*. In the case of *Eurytemora* the dimorphism is between the sexes, females having higher physiological and lower expressed genetic variation than males (Bradley, 1978a, b). Obviously in a species such as *Eurytemora* with obligate

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Abbreviations: LW, long wave temperature cycling (1° per week); SW, short wave temperature cycling (1° per day).

sexual reproduction, the underlying genetic variation must be the same in the two sexes, whether or not that variation is expressed.

The genetic and physiological variation observed in the two sexes can be considered as potentials for adaptation. Thus, females apparently have the greater capacity for internal adjustment to temperature change and males are more likely to be selected according to their genotypes. Such selection has been demonstrated using laboratory environments (Ketzner and Bradley, 1982). Populations maintained in the five different temperature environments, to be described later in this paper, diverged genetically. Genetic differences were also demonstrated between progeny of animals collected from intake and discharge waters of steam electric power plants (Bradley, 1978a; LaBelle and Bradley, 1982). However, variation in temperature, even over a wide range, is not sufficient by itself to produce genetic change. No genetic differences, but physiological differences were found between populations collected at different seasons in the wild (Bradley, 1982). Whether the genetic or physiological potential is the more important seems to depend on the rate rather than on the magnitude of the variation in temperature (Ketzner and Bradley, 1982).

When natural selection occurs, as it did in the five temperature environments just mentioned, we expect a reduction in genetic variation. We also expect a reduction in total phenotypic variation, of which the genetic variation is a part. Furthermore, to the extent that the potential for physiological change is important in successful reproduction, and assuming this potential varies genetically, we may find a reduction in the genetic variation in physiological potential or, in this case, in the ability to acclimate to higher temperatures.

The present paper examines phenotypic, genetic and non-genetic (largely physiological) variation in temperature tolerance of populations of *Eurytemora affinis* kept in five temperature environments for three years. Non-genetic variation is also examined as the response in temperature tolerance of individuals exposed to 23°C for 24 h. Genetic variation in this non-genetic response is also examined in the different environments.

Materials and Methods

The copepods used to stock the cultures initially were collected from Bear Creek, east of Baltimore Harbor in the Upper Chesapeake Bay, Maryland.

Environments and sampling

The temperature environments imposed on the various cultures were: constant at 10°C (10°C), constant at 15°C (15°C), constant at 23°C (23°C), cycling at 1° per day between 10 and 23°C (Short Wave or SW), and cycling at 1° per week between 10 and 23°C (Long Wave or LW).

The 23°C temperature was at or near the limit at which cultures could be maintained; the 10°C temperature was not the lowest possible temperature but allowed sufficient turnover of generations for selection to take place. Between 10°C and 4°C the generation time in *Eurytemora* increases from about one month to two months. The 15°C temperature, with generation time around three weeks, served as a control. Generation time is around 10 days at 23°C. The SW environment passed through the range of temperatures in as little as 13 days; thus, given the generation times at the various temperatures, most individuals were exposed to all temperatures. The LW environment, on the other hand, changed sufficiently

slowly, taking at least 13 weeks to cover the range, that individuals were exposed to only a fraction of the temperatures between 10°C and 23°C.

Each environment had approximately 40 ovigerous females initially. Samples of animals were withdrawn for testing directly or for raising progeny. At the time of sampling, the cycling environments were passing through 15°C, and the temperature was increasing. Progeny were raised at a constant (15°C) temperature, to isolate genetic differences among cultures, maternal influences on temperature tolerance having been shown to be minimal (Bradley, 1978a).

The assay for temperature tolerance

The assay for temperature tolerance was the same as used in Bradley (1978a). Temperature tolerance was based on the time, in minutes, until animals entered a coma after they were abruptly placed in 32°C water which was increased by 0.5°C every 5 min thereafter. Individual animals were placed in 2 ml of water in vials partially immersed, in racks, in an aquarium filled with water and heated by a thermostatically controlled heating-stirring unit. The assay was done on 12 animals at a time and usually took 30 min or less to complete, depending on when the last animal became comatose. Vials containing comatose animals were removed and the animals allowed to recover. This assay was shown to be an accurate predictor of survival at high temperatures, with the obvious advantages of being short-term and non-lethal (Bradley, 1976).

Temperature tolerance was measured on each animal before and after acclimation for 24 h at 23°C. Males and females were assayed separately, 12 in each run.

Measurement of genetic and non-genetic variation

Differences observed between copepods in temperature tolerance, or between individual organisms in any quantitative trait, may be the result of their different phenotypes or of their external or internal environments. Thus, the totality of differences or the variation among individuals, usually summarized statistically as the variance, can be partitioned into genetic and non-genetic variation. These two components may be further divided, giving four components of variance as follows:

$$\sigma_P^2 = \sigma_A^2 + \sigma_{NA}^2 + \sigma_{E_g}^2 + \sigma_{E_s}^2$$

where σ_P^2 = phenotypic variance, σ_A^2 = additive genetic variance (due to the sum of the individual gene effects), σ_{NA}^2 = genetic variance due to interaction between genes in pairs (dominance) or between pairs of genes (epistasis), $\sigma_{E_g}^2$ = the variance due to changes in the external environment, and $\sigma_{E_S}^2$ = the variance due to internal (physiological) changes in individuals. Obviously, the first two components together are the genetic variation and the latter two the non-genetic or environmental variation. The components of particular interest in this study were the σ_A^2 and $\sigma_{E_S}^2$ terms, representing, respectively, the potential for genetic change, since only individual genes and not parental gene combinations are transmitted, and the potential for physiological change.

Estimates of σ_A^2, the additive genetic variance, referred to also as genic variance, were obtained from the variance among broods, estimated from analysis of variance. Since one-half the additive genetic variance in a population is among broods and the other half within broods, the variance among broods is an estimate of one-half of the additive genetic variance.

Using full-sibs instead of half-sibs to estimate genic or additive (as opposed to total genetic) variance seemed justifiable since there is little sperm storage and no maternal and non-additive genetic variation was observed in temperature tolerance in *Eurytemora* (Bradley, 1978a). Both of these components of variation would contribute to the variance among broods and thus inflate the estimates of additive genetic variance. The sampling variance (and hence the standard deviation) of each estimate was derived from the analysis of variance by the method described in Brownlee (1965). Further details of the methods of analyses appear in Bradley (1978a).

Physiological variation, the other component of variation in temperature tolerance which was of particular interest in this study, was measured in two distinct ways. One estimate was obtained by subtracting the estimate of additive genetic variance from its corresponding phenotypic variance. The estimate of $\sigma_{E_S}^2$ was thus: $\sigma_P^2 - \sigma_A^2$. The justification for this estimate was that non-additive genetic variance (σ_{NA}^2) and variance due to general environmental effects ($\sigma_{E_g}^2$), at least in a constant environment, were both found to be negligible, as was stated earlier.

The second estimate of physiological variation was obtained by exposing individuals to a high temperature (24°C) for 24 h, measuring temperature tolerance before and after the exposure. Previous work (Bradley, 1978b) had shown that the 24°C temperature was sufficient to induce dramatic shifts in temperature tolerance but without mortality. Although acclimation was not complete at 24 h there was little interaction (in particular change in ranking of individuals) as animals changed in tolerance over the next 24 h or 48 h period. Thus, the short-term change could be assumed to represent the relative potentials of individuals for physiological or individual adjustment.

Since tolerances were measured before and after the acclimation just described, two further sets of measurements were possible. First, additive genetic variance in temperature tolerance after acclimation could be estimated, just as it was measured before acclimation. Second, the additive genetic variance in the degree to which individuals acclimated could also be measured. We realize that this variance is a function of the variances in temperature tolerance before and after acclimation and the covariance between them.

Additional data on fitness traits

Other data were collected on individuals and broods sampled from the environments. Egg production in a female was estimated by isolating an ovigerous female in a droplet of water and counting the eggs in her egg sac. Sex ratio was measured as the ratio of males to the total number of adults in each brood, viability as the number of adults expressed as a fraction of the number of eggs.

Finally, in order to relate temperature tolerance and other fitness traits by brood, data were collected on egg production, viability and sex ratio as well as on temperature tolerances of male and female progeny, the latter to measure average tolerances in each brood. These animals were wild collected, not derived from the five environments.

Results

Data on genetic and non-genetic variation on temperature tolerances of progeny from the five temperature environments were collected over a six month period after one year exposure of cultures and again after three years. The data from the

two collection periods and from the two sexes of progeny are reported separately in each of the tables.

The phenotypic variances in temperature tolerance shown in Table I suggest effects of acclimation, sex and culture environment. In the first set of data (after 1–1½ years) females were more variable than males, but after three years the variances were quite comparable. In the first set of data both sexes increased in variance after acclimation by comparable amounts, but in the later tests, after three years, female variances increased much less than did male variances following acclimation, and in some cases actually decreased.

Comparing phenotypic variances among the environments did not reveal any evidence of selection, with the possible exception of the lower variance of male progeny from the 23°C environment after three years. Note that the 10°C and 23°C variances in male progeny changed in opposite directions, before and after acclimation, between the first and second measurements.

The additive genetic variances in male progeny listed in Table II provide evidence for selection. They were lower in the SW and LW environments in the earlier data and in the 23°C, SW and LW environments in the later data. In these same environments the male variances were less than the corresponding female variances, suggesting that the genetic consequences of selection were different in males and females. The expression of additive genetic variance generally increased following acclimation, by about 100% in the first set of measurements and by about 30%, on average, in the second.

Estimates of physiological variance are shown in Table III, obtained by subtracting the additive genetic variances from their corresponding phenotypic vari-

TABLE I

Phenotypic variances in temperature tolerance before and after acclimation in male and female progeny from five environments on two occasions.

Environment	Preacc.	Postacclimation	Preacc.	Postacclimation
After 1–1½ years		Male		Female
I 10°C	18.0	31.5	26.5	33.9
23°C	25.4	41.9	25.4	34.3
II SW	21.1	35.7	29.3	46.6
15°C	24.5	42.8	25.4	53.2
III LW	14.4	33.5	16.9	44.3
15°C	13.2	43.5	13.9	37.5
			(240 animals per estimate)	
After 3 years				
10°C	33.1	30.8	28.8	50.5
15°C	20.8	30.2	23.5	16.5
23°C	14.0	26.3	26.8	25.6
SW	21.7	41.0	24.3	37.3
LW	18.0	50.4	21.7	25.1
			(80 animals per estimate)	

The 10°C, 15°C and 23°C were constant environments; the SW and LW cycled at 1°/day and 1°/week between 10° and 23°C. The first experiment was done with three pairings of environments over six months.

TABLE II

Estimates, with standard errors, of additive genetic variance in temperature tolerance before and after acclimation in male and female progeny from five environments.

	Envir.	Preacc.	Postacclimation	Preacc.	Postacclimation
After 1–1½ years			Male		Female
I	10°C	15.1 (1.3)	31.5 (3.6)	13.5 (2.1)	22.7 (3.0)
	23°C	15.5 (1.4)	10.9 (2.0)	14.2 (2.2)	16.8 (2.3)
II	SW	5.7 (1.1)	16.8 (2.0)	20.5 (2.2)	45.7 (4.2)
	15°C	21.6 (2.0)	33.4 (2.3)	9.9 (1.8)	33.0 (3.9)
III	LW	5.2 (0.9)	28.1 (2.9)	2.7 (0.9)	23.9 (3.5)
	15°C	2.5 (0.7)	28.3 (2.2)	5.7 (1.0)	9.0 (2.0)
			(240 animals in 60 broods per estimate)		
After 3 years					
	10°C	21.0 (4.4)	13.0 (3.7)	14.9 (3.6)	17.0 (5.8)
	15°C	10.4 (2.6)	11.2 (3.5)	0.0 (2.2)	2.9 (1.7)
	23°C	1.5 (1.4)	1.0 (2.6)	7.1 (2.9)	11.9 (3.1)
	SW	1.7 (2.2)	14.0 (4.7)	7.7 (2.8)	7.1 (4.0)
	LW	0.0 (1.7)	17.0 (5.8)	8.2 (2.5)	4.1 (2.6)
			(80 animals in 20 broods per estimate)		

The 10°C, 15°C and 23°C were constant environments; SW and LW cycled at 1°/day and 1°/week between 10° and 23°C. The first experiment was done with three pairings over a six month period.

TABLE III

Estimates of physiological variation in temperature tolerance in male and female progeny in five environments, by subtraction of genetic variance from phenotypic variance.

	Envir.	Preacc.	Postacclimation	Preacc.	Postacclimation
After 1–1½ years			Male		Female
I	10°C	2.9	0.0	13.0	11.2
	23°C	9.9	31.0	11.2	17.5
II	SW	15.4	18.9	8.8	0.9
	15°C	2.9	9.4	15.5	20.2
III	LW	9.2	5.4	14.2	20.4
	15°C	10.7	15.2	8.2	28.5
				(240 animals per estimate)	
After 3 years					
	10°C	12.1	17.8	13.9	33.5
	15°C	10.4	19.0	23.5	13.6
	23°C	12.5	25.3	19.7	13.7
	SW	20.0	27.0	16.6	30.2
	LW	18.0	33.4	13.5	21.0
				(80 animals per estimate)	

The 10°C, 15°C and 23°C were constant environments; SW and LW cycled at 1°/day and 1°/week between 10° and 23°C.

ances, assuming, as explained earlier, that the other two components of variance in temperature tolerance can be ignored, at least in constant temperatures. These estimates also include errors of estimation of the two variances from which they are derived. There is evidence of an effect of acclimation but little systematic effect of either culture environment or sex. Male progeny from the three year sampling from each environment were always more physiologically variable after acclimation. This was not the case for male progeny from the earlier sampling nor for female progeny at either sampling. However, in most instances physiological variation was greater in progeny after acclimation than before.

In two of the environments (SW and LW) the effect of acclimation on physiological variation was opposite in the two sexes at the 1–1½ year sampling, and opposite in two other environments (15°C and 23°C) at three years. The effects of acclimation at 1–1½ years and at three years can be compared for each sex and each environment. In more cases than not the effect of acclimation was reversed, particularly in the female progeny. For example, female progeny from the 23°C environment were more physiologically variable after acclimation in the early sample but less physiologically variable after acclimation in the later sample.

Physiological variability was also measured as the change in mean tolerance due to acclimation to a higher temperature (23°C) for 24 h. The average responses of progeny to the higher temperature are shown in Table IV. Again the sexes were comparable, environment by environment, in each sampling period. Exceptions were in the SW environment in both periods. In the earlier sampling male progeny from the SW regime acclimated less than female progeny, but the order was reversed, presumably due to further selection, in the later sampling. The responses of male and of female progeny at three years were lowest in the SW environment and were actually the two lowest of all ten mean responses.

TABLE IV

Average responses of individual progeny from five environments to acclimation at 23°C for 24 h, measured as difference in temperature tolerance before and after acclimation.

Environment		Male	Female
After 1–1½ years			
I	10°C	5.6 (3.7)	5.5 (4.6)
	23°C	5.9 (4.3)	6.2 (4.7)
II	SW	4.8 (4.3)	7.6 (4.4)
	15°C	4.0 (3.9)	7.3 (5.4)
III	LW	5.4 (4.2)	4.6 (5.1)
	15°C	4.4 (4.1)	3.7 (5.3)
		(240 animals per estimate)	
After 3 years			
	10°C	9.3 (5.1)	9.7 (6.6)
	15°C	8.9 (5.2)	9.6 (6.1)
	23°C	10.4 (4.7)	10.3 (6.1)
	SW	8.2 (6.1)	6.0 (5.9)
	LW	9.2 (7.4)	10.5 (5.3)
		(80 animals per estimate)	

The 10°C, 15°C and 23°C were constant environments; SW and LW cycled at 1°/day and 1°/week between 10° and 23°C. Standard deviations (not standard errors) are shown in parentheses.

Physiological variability or flexibility, measured as just described, itself varies genetically among individuals. Differences among individuals in their potential for physiological change are due partly to their different genotypes. Additive genetic variances in physiological flexibility are shown in Table V. There apparently were effects of sex, of time of sampling, and of environmental treatment.

Female progeny had more additive genetic variance in flexibility than did males at the 1–1½ year sampling but not at three years. Variances were lower at three years, with one exception. In the SW environment the variance in male progeny increased. Variance in the 15°C environment, on the other hand, had almost disappeared in both sexes at three years. In both the early and later samples genetic variance in flexibility in male progeny from the 23°C environment was not detected.

Data on other traits, as well as on mean temperature tolerances, as affected by the regimes, are shown in Table VI which is a summary of data given in more detail in Bradley (1982). In general, differences between animals randomly sampled from the regimes did not persist in progeny. Hence the differences were presumably largely environmental, specifically maternal. Egg production was lowest in the 23°C environment, intermediate in the SW and LW regimes. The animals in these latter regimes were all exposed to 23°C. Egg production levels among female progeny raised at 15°C did not differ between regimes.

Sex ratio is an important trait in that a higher proportion of males would decrease overall reproduction, given similar reproduction levels in females. The percentage of males was significantly greater in broods from cycling regimes than from the 23°C regimes. The greater proportion of females in 23°C would not be sufficient to compensate for the lower egg production in 23°C, assuming these figures are indicative of egg production and sex ratios in nature. The differences in sex ratio did not occur in progeny, so presumably were not the result of natural selection.

Viability, also an important component of fitness, is lowest in 23°C. The total number of broods sampled was also low in the 23°C regime, but of that total a higher proportion had no progeny than was the case in other regimes. The lower

TABLE V

Estimates, with standard errors, of additive genetic variance in degree of acclimation (when exposed to 23°C for 24 h) in male and female progeny from five environments on two occasions.

Environment		Males	Females
After 1–1½ years			
I	10°C	9.8 (1.4)	12.1 (2.0)
	23°C	0 (0.9)	9.9 (1.7)
II	SW	6.4 (1.4)	10.4 (1.5)
	15°C	5.8 (1.1)	11.8 (2.2)
III	LW	9.2 (1.5)	15.8 (2.4)
	15°C	6.1 (1.3)	6.3 (1.5)
After 3 years			
	10°C	4.9 (2.1)	7.8 (3.6)
	15°C	1.9 (2.0)	0 (2.4)
	23°C	0 (1.6)	6.3 (3.1)
	SW	16.3 (4.6)	2.1 (2.6)
	LW	6.6 (4.6)	3.1 (5.7)

The 10°C, 15°C and 23°C were constant environments; the SW and LW cycled at 1°/day and 1°/week between 10° and 23°C.

TABLE VI

Temperature tolerance of male and female progeny, egg production, sex ratio and viability in five temperature environments.

		10°C	15°C	23°C	SW	LW
Tolerance 0		17.3	16.2	16.7	17.4	15.8
Tolerance +		20.3	20.7	19.8	23.0	18.8
Animals per mean		80	80	80	80	80
Egg production:	Parents	33.1	33.6	11.1	24.3	24.9
	F_1	24.4	24.7	23.6	25.1	25.2
Sex ratio:	Parents	32.9	39.4	33.3	48.7	43.2
	F_1	39.1	48.1	43.2	46.9	42.9
Viability:	Parents	50.9	47.8	27.2	44.3	55.1
	F_1	30.9	36.1	30.0	33.5	37.5
No. of broods:	Parents	118	136	12	153	115
	F_1	41	47	31	39	46

The 10°C, 15°C and 23°C were constant environments; the SW and LW cycled at 1°/day and 1°/week between 10°C and 23° (Adapted from the original data in Bradley, 1982).

viability in 23°C broods did not persist to the F_1 generation, although broods originating in the 10°C and 15°C cultures differed significantly in viability.

The relationships between temperature tolerance and other fitness traits in the same broods were investigated with data collected for heritability estimates (Bradley, 1978a). The correlation matrix is shown in Table VII. There is little obvious evidence of negative relationships between temperature tolerance and egg production, viability, or sex ratio. Indeed the relationship between egg production and tolerance, free of viability effects, was actually positive. The standard partial regression coefficients (path coefficients) between tolerance and egg production were 0.48 and 0.21 for male and female brood averages, respectively. On the other hand, if the correlation between sex ratio (proportion of males) and tolerance is real, then the correlation with proportion of females, and so with reproductive output, would be negative.

DISCUSSION

There is some evidence that directional selection for temperature tolerance in the 23°C environment resulted in a reduction in genetic (and phenotypic) variation. The estimates of genetic and phenotypic variances in male progeny from the 23°C environment were lower than in the other environments at three years (Tables I and II). This agrees with the expectation that additive genetic variation will decrease as certain genotypes leave relatively more progeny and gene frequencies move away from intermediate levels. McLaren (1976) reported reductions in genetic variance in a number of demographic traits in the copepod *Eurytemora herdmani* as a result of selection.

On the other hand, in the cycling environments (SW and LW), where selection was actually more effective than in the 23°C environment according to the mean tolerances of randomly sampled progeny measured at two years (Ketzner and Bradley, 1982), additive genetic variance appeared to be maintained in males at the

TABLE VII

Correlations among number of eggs, number of adults, egg-to-adult viability, sex ratio, average tolerance of male progeny and average tolerance of female progeny in 51 broods.

	2	3	4	5	6
1. Egg no.	0.48	−0.39	0.02	0.11	0.39
2. Adult no.		0.44	0.12	0.18	0.24
3. Viability			0.27	0.17	0.04
4. Sex ratio				0.23	0.21
5. Mean tolerance[a] of female progeny					0.28
6. Mean tolerance[a] of male progeny					

[a] Measured as least squares deviation of female brood mean tolerance, distinct from male parent effect.

three year sampling. However, acclimation for 24 hours was necessary for the variation to be expressed (Table II).

Genetic variation in the female progeny from the various environments was maintained and expressed, with the possible exception of those from the 15°C environment at three years (Table II). These results contrast with previous results from progeny of wild populations, where female progeny consistently expressed much lower genetic variation than did male progeny (Bradley, 1978a). Such a change may have resulted from selection, or perhaps, although this is not a likely explanation, from inbreeding in the laboratory cultures.

The association of environmental and genetic variation has been demonstrated before in Mendelian traits such as enzyme variants (McDonald and Ayala, 1974), third chromosome inversions (Dobzhansky *et al.*, 1966) and coat color (Gill, 1977), and in quantitative traits such as skeletal development in mice (Garrard *et al.*, 1974) and bristle number in *Drosophila* (Beardmore, 1961; Gibson and Bradley, 1974).

Thus, there are precedents for exhaustion of genetic variance in a constant environment and its maintenance in a cycling environment, always assuming that temperature tolerance is under selection. Such an assumption seems valid given previous work demonstrating that temperature tolerance measured by the short-term assay used in this study is closely related to survival at high temperatures (Bradley, 1976).

The ability to alter temperature tolerance internally may also be important to survival. In the past we have shown that female *Eurytemora* can acclimate to higher temperatures to a markedly greater degree than can males (Bradley, 1978b). Females and males acclimated at 23°C for 24 h could be easily classified, without error, solely on their temperature tolerances. Such a clear sexual dimorphism was not observed in general in the present experiment, but neither were the responses of the two sexes generally the same in the different environments.

Two measures of physiological flexibility were used in this study. The measurements shown in Table III reflect random non-genetic variation in a constant 15°C environment and may not be related to the potential of the organism to adapt physiologically. The measurements in Table IV indicate the degree to which individuals can adapt in the short-term (24 h). In previous work both estimates were much higher in females (Bradley, 1978a,b) but this was not the case in the present study. In general, males and females were more alike in short-term change in mean tolerances (Table IV) than in their random physiological variances (Table III). Also, while the degree of acclimation was generally higher after three years (Table

III), neither total phenotypic (Table I) nor physiological variation (Table III) increased as much after acclimation at three years as either did at 1½ years. Thus it appears that random physiological variation and physiological response to stress are not related, at least at the population level.

There was evidence for selection for average response due to acclimation, the second measurement, when the figures at 3 years and 1½ years were compared (Table IV). The exception was the SW environment. The sexes were comparable in most environments, again except in the SW environment. Additive genetic variance seemed to be absent in male progeny at both samplings in the 23°C environment, and low in the 15°C environment after three years in both males and females. This may indicate a significant response to selection since the levels of acclimation (Table IV) were also high in the 23°C and relatively high in the 15°C environment. In males, but not in females, the average responses at three years and the additive genetic variances at three years were in reverse order by environment, again suggesting selection for physiological flexibility in males.

Why the sexes should behave differently in their response to selection is not clear, especially since in a sexually reproducing species there is 50% gene flow or exchange between the sexes each generation. One of the interesting features of this experiment is that the sexual dimorphism in expressed genetic and physiological variance disappeared, to be replaced by apparent dimorphisms in selection response and in the effect of acclimation on expressed variance.

The fact that the consistently higher additive genetic variance in males and consistently higher responses to acclimation in females were no longer observed has been discussed earlier. Some of the differences in selection response have also been mentioned, with more evidence for selection in male progeny. Further evidence that selection pressure is greater on males, or at least on genotypes expressed in males, comes from the genetic variances measured before acclimation in the 23°C, LW and SW environments (Table II), which were all low.

The effect of acclimation on the expressed variance in temperature tolerance also differed between the sexes, particularly in certain environments. Whereas acclimation increased phenotypic variation in temperature tolerance in all environments in both sexes at 1–1½ years, at the 3 year sampling the effects were opposite in the sexes in the 10°C, 15°C, and 23°C environments (Table I). Genetic variances at those years (Table II) were increased following acclimation in the 1–1½ year samples, but at 3 years the effect in the SW and LW environment was quite different in males and females. Incidentally, Ushakov *et al.* (1977) suggested that genetic distinction is reduced following acclimation. So our evidence both contradicts and supports this suggestion, dependent partly on whether the animals have been selected or not.

All of this again begs the question of how the sexual dimorphism in genetic variation and physiological variation is maintained in nature and how it was reduced, reversed, or even eliminated in the laboratory. The large residual genetic variances observed would seem to eliminate random drift in gene frequencies as an explanation, so the change must have occurred by selection and clearly by selection pressure quite different from that in nature.

Differential selection pressure between the sexes is one means by which genetic variance and the sexual dimorphism might be maintained (Bradley, 1982). However, the present data indicate that the former was maintained (or expressed) in females, but less so in males; and the latter changed in that males no longer expressed more genetic variance and actually expressed less than did females. Thus we could conclude that the dimorphism itself has genetic variation.

Another model, also suggested in the review (Bradley, 1982) states that direc-

tional selection for temperature tolerance occurs in the warmer temperatures and this selection is resisted by negative relationships with other fitness traits, such as egg production. Thus, as temperatures decreased, tolerance would decline because of selection for other traits. Egg production was lowest in the environments reaching 23°C (Table VI) in which tolerance was highest. However, the differentiation was not present in the F_1 progeny. Greater proportions of males were present in the SW and LW regimes, but again the difference disappeared in the progeny. Viability was lower in 23°C than in the cycling regimes and lower in the SW than in the LW regime. However, once again these differences were not present in the F_1 progeny. In summary, there are no obvious mean genetic differences between regimes in fitness traits other than temperature tolerance, but environmental effects on these traits may result in selection for temperature tolerance being less effective. Finally, there are no obvious relationships between mean tolerances and mean egg production, sex ratio or viabilities of progeny in the regimes.

There are no dramatic negative relationships between temperature tolerance and other fitness traits at the brood level either. In Table VII correlations are shown between temperature tolerances and four fitness traits. There is no evidence directly from the correlation matrix on negative relationships, nor when one performs a path analysis including egg production, viability and mean tolerance. In both male and female progeny the standard partial regressions of tolerance on egg production are positive (0.48 and 0.21 in males and females, respectively). If the relationship had been negative and genetic, then selection for tolerance would in effect be reversed when temperature stress declined, a form of genetic homeostasis proposed a long time ago by Lerner (1954). The argument by Lerner was in the context of artificial selection (being resisted by natural selection). The argument here would have been for a return of genes for high temperature tolerance to intermediate frequencies as a result of the highest fitness at lower temperatures of genotypes having intermediate temperature tolerances. So far, therefore, this model is not supported, although it should be noted that there may be a negative relationship between proportion of females and temperature tolerance at the brood level.

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